suggesting that they reflect to some extent, distinct biological processes.
Aim: to investigate whether Helix-II and CTX-II reflect distinct degradation mechanisms, prevailing differently in distinct areas of cartilage, and varying differently with age and menopausal status in healthy subjects.
Methods: Immunohistochemistry was performed on full depth knee cartilage biopsies obtained from OA patients, and the tissue distribution of Helix-II and CTX-II immunoreactivity was determined. Urinary Helix-II and CTX-II were measured in 105 healthy women (from 26 to 90 years, mean: 59 years) and 48 healthy men (from 26 to 79 years, mean: 48 years).
Results: Immunoreactivity for Helix-II, but not for CTX-II, was associated with the matrix of fibrillated articular surfaces. Adjacent non-fibrillated surfaces were negative. Immunoreactivity for CTX-II, but not for Helix-II, was associated with matrix close to bone outgrowths into cartilage, often in areas of calcified cartilage. Urinary levels of Helix-II did not change significantly with age or menopausal status in healthy women. Urinary levels of CTX-II were stable in healthy premenopausal women from age 35 to menopause, but in contrast to Helix-II, increased markedly by an average of 70% ($p<0.0001$) after the menopause and then increased slightly but not significantly with age in postmenopausal women. In healthy men, there was no significant change with age both for urinary Helix-II and CTX-II.
Conclusion: These differences in cartilage tissue distribution and relative urinary levels of Helix-II and CTX-II (i) support the existence of distinct mechanism of type II collagen breakdown during cartilage degradation; (ii) show that the prevailing breakdown mechanism varies with the region of cartilage; and (iii) show that these mechanisms differ in their susceptibilities to age and menopause. Helix-II and CTX-II may provide distinct and complementary information on cartilage turnover.

P65

INHIBITION OF TYPE II COLLAGEN DEGRADATION IN THE OVARIECTOMIZED RAT BY ESTROGEN AND A SELECTIVE ESTROGEN RECEPTOR MODULATOR: CORRELATION BETWEEN TYPE II COLLAGEN BIOMARKERS

PG Mitchell, MG Chambers, C Lin, D Cummins, HW Cole, RL Cain, L Ma, MJ Berna, CA Schmalz, BL Ackermann, KL Duffin, JA Dodge, HU Bryant
Musculoskeletal Division, Eli Lilly and Company, Indianapolis, IN

Aim of Study: To determine the effect of estrogen and a SERM (LY505960) on the type II collagen biomarkers CTX-II and NET2C, in the rat ovariectomy (ovx) model and to investigate correlations between these two biomarkers.
Methods: Forty-two 6-month-old female virgin Sprague Dawley rats underwent ovx and an additional 6 animals were subjected to sham ovx. Daily dosing began on day four post-ovx and consisted of the following eight groups (six animals/group): sham/vehicle, ovx/vehicle, ovx/17a-estradiol @ 0.01 and 0.10 mg/kg, ovx/LY505960 @ 0.01, 0.10, 1.0 and 10.0 mg/kg. Prior to sacrifice (32 days post-ovx), overnight urines were collected. Urine samples from each animal were analyzed for type II collagen fragments containing the C-terminal telopeptide-derived peptide EKGPDP (CTX-II, Cartilaps: Nordic Bioscience Diagnostics A/S) by competition ELISA. The assay for a type II collagen neoepitope (NET2C) utilized online immunoaffinity capture with an antibody (9A4) specific for the collagenase-generated C-terminal neoepitope, followed by LC/MS/MS to quantify a specific 14 mer collagen peptide (DGPSGSDGPPGPQG). CTX-II and NET2C concentrations in urine were normalized to creatinine levels.
The data for each analyte were converted into pmoles/mole of creatinine and a Box-Cox transformation carried out in order to stabilize variances between groups to comply with standard statistical ANOVA assumptions. A comparison of means was performed on the transformed data using Dunnett's test for comparing each group to the non-dosed ovx group.
Results: At 32 days post-ovx the CTX-II concentrations had increased 4.5 fold in the ovx animals while NET2C levels had increased 3.3 fold, compared with the sham-ovx animals ($p<0.01$). In comparison to the untreated ovx animals, both doses of 17a-estradiol and all doses of LY505960 significantly reduced both CTX-II levels and NET2C levels ($p<0.01$). A correlation coefficient of 0.889 (regression $R^2 = 0.7904$) was obtained when the day 32 creatinine-adjusted values of CTX-II and NET2C values were plotted against each other. There was a small but statistically significant ($p=0.0003$) shift in the linear regression relationship (CTX-II intercept at 0.69) and, a matched pairs analysis demonstrated that every treatment group except the sham-ovx group had higher CTX-II than NET2C values.
Conclusions: We have extended previous reports relating to CTX-II in the ovx model, to demonstrate that NET2C, a collagenase-derived C-terminal type II collagen neoepitope, is elevated in ovx rats and is sensitive to treatment with either estrogen or the SERM, LY505960. Experimentally, a good correlation between CTX-II and NET2C was observed suggesting that release of these two-type II collagen epitopes are temporally and mechanistically linked. The robust inhibition of type II collagen degradation by LY505960 in the rat ovx model suggests that this compound may be evaluated for treating osteoarthritis in postmenopausal women.

P66

ADAMTS-7 DEGRADES COMP IN A Zn^{2+}- AND pH-DEPENDENT MANNER AND IS SIGNIFICANTLY UPREGULATED IN THE CARTILAGE AND SYNOVIUM OF PATIENTS WITH RHEUMATOID ARTHRITIS

C Liu, K Ilalov, B Sehgal, PE Di Cesare
Orthopaedic Surgery, NYU-Hospital for Joint Diseases, New York, NY

Study aims: Our genetic screen led to the discovery of ADAMTS-7 as an COMP-binding protein and ADAMTS-7 binds to the EGF domain of COMP via its C-terminal four TSP motifs. The purpose of this study was to characterize the mechanism underlying the ADAMTS-7-mediated COMP degradation and determine whether ADAMTS-7 expression was altered in the arthritic conditions.
Methods: *In vitro digestion assay:* purified COMP (250nM) was incubated with recombinant ADAMTS-7 (25nM) in buffer (50mM Tris-HCl, 100mM NaCl, pH 7.5) supplemented with 5mM $CaCl_2$, 2mM $ZnCL_2$, 2.5mM $MgCl_2$, or various combinations at 37°C for 12 hr. The digested proteins were visualized with Coomassie Blue R250. The same digestion was also performed at various pH values.
Subjects: Normal cartilage and synovium were obtained from the knees of four patients who died of diseases unrelated to arthritis. OA cartilage was obtained from the distal femora of 8 patients and RA cartilage and synovium from the knees of 4 RA patients. *Expression of ADAMTS-7 in arthritic tissues:* Real-time PCR was performed using a sequence-specific probe and primers for ADAMTS-7. 18s rRNA was used as internal control. PCR reactions were performed with 5 ng of cDNA, 100 nM probe, 200 nM each primer, and 10.0 μl of TaqMan Universal 2' PCR Master Mix in a 20-μl reaction volume.
Results: *Zn^{2+} is essential for the cleavage of COMP by ADAMTS-7* – A degraded COMP fragment was detectable in the digestion buffer with Zn^{2+}but was undetectable in the digestion buffer with Ca^{2+} or Mg^{2+} used alone. In the presence of Zn^{2+}, the addition of Ca^{2+}, but not Mg^{2+}changed the electrophoretic